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ENVIRONMENTAL RESERVOIRS OF MYCOBACTERIUM AVIUM SSP. PARATUBERCULOSIS ON BEEF COW-CALF FARMS

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"ENVIRONMENTAL RESERVOIRS OF MYCOBACTERIUM AVIUM SSP. PARATUBERCULOSIS ON BEEF COW-CALF FARMS"

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SUMMARY:

This study was conducted in order to gain a greater understanding of the environmental factors affecting the management of Johne's disease in beef cow-calf herds in Western Canada. The first part of the study consisted of recruiting six Johne's disease positive cow-calf herds from Saskatchewan that were studied quarterly for one year in order to: 1) identify the environmental areas including water sources on a cow-calf farm that might be contaminated with *Mycobacterium avium subspecies paratuberculosis*, (*Map)*, 2) determine if some species of wildlife commonly found on cow calf farms become infected with *Map*, and 3) establish if there are seasonal influences that affect either of these two potential sources of infection. Only 2 of 418 (0.5%) environmental samples and 0 of 438 (0%) wildlife samples collected were positive for *Map* on culture with no significant difference found between farm prevalence or season. The positive samples were collected from a dugout and a calving pen. After 5 months no viable *Map* was detected at these sites.

The second part of the study also attempted to identify areas of environmental contamination with Map during the calving season and attempted to determine the value of using environmental sampling as an alternative to herd testing. Thirty beef cow-calf herds with a history of Johne's disease from Manitoba, Saskatchewan, and Alberta were recruited to participate. On each farm, cattle were tested with pooled fecal culture (5 cattle per pool) and approximately 15 environmental samples from each farm were collected and cultured during the calving season. The herd prevalence was compared to the environmental results to find any potential relationship. 27/30 (90%) of herds selected completed all the required sampling and were included in the analysis. Of these

27 herds, 17 (63.0%) had at least one positive pooled fecal culture. None of the 114 water samples (0%) and 15/243 (6.2%) of the environmental samples (non-water) were positive for *Map*. Only 8 of the 27 (29.6%) herds had at least one environmental sample test positive for *Map*. Of the 17 herds identified as actively positive for Johnes disease by pooled fecal culture, only 6 (35.3%) were also positive on environmental sampling. Of the 10 herds that were negative on pooled fecal cultures, 2 (20%) were positive on environmental sampling. The agreement between the two testing methods had a calculated kappa of 0.13 which suggests only slight agreement. The level of environmental contamination on cow-calf farms in Western Canada at this time is quite low as shown by only 8 of 27 herds in this study with any positive sites detected. While reducing the cost of testing, using environmental samples as done in this study is not sensitive enough as a herd test to be used in place of traditional herd test methods.

The third part of the study was designed to establish the ability of the *Map* bacteria to survive the climate of a Western Canadian farm. Initially three sites were identified as culture positive for *Map* and once confirmed in the laboratory these sites were re-cultured until multiple negative culture results were attained. However, there were too few environmentally positive test that could be protected from recontamination. No sites remained culture positive 5 months past the initial positive culture. To improve on these results an experimental field study was designed to evaluate the persistence of *Map* in the environment. Four sites were selected and intentionally contaminated with manure from a cow with clinical Johne's disease. These sites were regularly cultured until three consecutive negative cultures were attained. We are still awaiting final culture results for this phase of the study.

INTRODUCTION:

Johne's disease, caused by *Mycobacterium avium subspecies paratuberculosis*, (*Map*), is a progressive and debilitating disease of livestock affecting beef cattle in Western Canada. It causes a profuse, untreatable diarrhea with high levels of bacterial shedding, dramatic weight loss, and eventually death. The primary modes of transmission include either direct exposure through the fecal-oral route, through contact with a contaminated environment or through consumption of infectious colostrum or milk (Giese and Ahrens, 2000). Calves from cow-calf herds, unlike dairy herds, cannot be isolated immediately after birth and so control efforts must focus on culling infected cattle and reducing exposure to the bacteria from environmental sources. Environmental contamination on cow-calf herds has not yet been studied but may be vital for the successful management of this disease. *Map* has been shown to be able to survive well in various environmental conditions (Whittington et al., 2004). The level of environmental contamination is dependent on the persistence of the bacteria in the environment as well as the degree of recontamination from cattle or other potential carrier species. Multiple regions around the world and within Canada have proactively developed Johne's control programs due to the concern that Johne's disease may be involved with Crohn's disease in humans (Chiodini et al., 1984; Collins and Manning, 1995; Hermon-Taylor, 2000; Manning, 2001). The process of controlling Johne's disease involves repeated testing of the herd, culling affected animals, and cleaning up the environment prior to releasing new animals onto the previously contaminated areas. This is an expensive and difficult

process. If the environmental contamination persists, control of this disease may never be achieved. This would result in economic losses due not only to lost production but also to the cost of repeatedly testing the herd, lost use of contaminated areas, and potential lost markets for cattle. Environmental testing has been suggested as an alternative to the traditional herd test methods for the dairy industry and could reduce the cost of testing a herd if it can replace individual animal testing (Raizman et al., 2004).

Water quality has always been important in disease control. Contaminated water acts as a potential source of infection for cattle, other animals and humans as well. Contaminated water could potentially move downstream as well as into the groundwater causing further environmental and health concerns. In studies done in Europe, Australia, and the United States of America various carnivores, rodents, and birds were found to either be infected with or spreading the bacteria(Beard et al., 2001; Ayele et al., 2001; Corn et al., 2005). These alternate species could undermine the control efforts attempted on affected farms if not included in the management plan. Understanding which species contribute to the level of *Map* contamination on a farm can help adjust management practises in order to reduce this risk. There has been remarkably very little research done on controlling *Map* infections in beef cow-calf herds under Western Canadian conditions. The vast majority of research on Johnes disease has focussed on controlling the disease in dairy herds.

The objectives of this study were to:

1. Identify the environmental areas including water sources on a cow calf farm that become contaminated with *Mycobacterium avium ssp. paratuberculosis (Map).*

2. Determine and compare which common wildlife species on cow-calf farms contribute to the spread and persistence of *Map.*

3. Assess the potential of environmental sampling as an alternative to fecal testing on cow calf farms.

4. Formulate informed recommendations regarding the environmental, wildlife, and water quality concerns for the control of Johne's disease on cow calf farms in Western Canada.

METHODS:

Herd Recruitment:

In the summer of 2005, approximately 30 beef cow-calf herds from Western Canada were identified as being infected with Johne's disease. Herds were recruited through contacts with local veterinarians and producers were asked if they wished to participate in the study. An infected herd was considered a herd that had clinical cases of Johne's disease diagnosed by their veterinarian within the last 2 years and at least one positive laboratory test confirmation. Herds were selected due to a willingness to participate and accessibility of cattle for sampling. The selected herds were tested using pooled fecal culture to determine the prevalence of *Map* infections. Six herds were selected for the Environmental and Wildlife Seasonality portion of the study and 30 herds (including the previous 6) were selected for the Environmental Testing during calving season part of the study.

Part One: Environmental and Wildlife Seasonality

Six farms were selected for this one-year study. On these farms, blood samples were taken from approximately 100 cattle greater than 2 years of age in the fall of 2005, and were tested with a ParaChek Enzyme Linked Immunosorbent Assay (ELISA) for *Map* antibody titres by the Animal Health Monitoring Lab of the Abbotsford Agriculture Centre. Pooled fecal cultures (five cattle per culture) were also done on these herds to enable comparison with both the serological and environmental sampling results. Four rounds of environmental sampling were conducted over the span of one year (one round of sampling every three months). To determine the distribution of environmental

contamination, 15 environmental samples were taken per sampling round from similar sites at each selected farm including calving areas, feeding areas, and water samples. Water samples were collected from livestock waterers, dugouts, ditches, and streams when present. Water sources and runoff areas were cultured to determine their significance to both on-farm transmission and to broader environmental contamination leading to the potential spread to locations downstream.

Each non-water environmental sample was collected by combining approximately 15ml of sample from 4 locations per site for a total of approximately 60ml of combined sample per site. Water samples consisted of 500ml of water collected from a site. This water was centrifuged at 1000g for 30 minutes and the resulting sediment was collected and sent for culture. Biofilm samples were collected at water sources by taking a sterile gauze pad and wiping the entire interior surface of the waterer. Environmental and pooled fecal samples were frozen until being submitted for culture after each round at the Animal Health Monitoring Lab in Abbotsford, British Columbia where they were stored frozen until culture. The modified BACTEC 12B culture method was utilized for detecting *Map* from these samples. All positive cultures were tested using the IS900 polymerase chain reaction (PCR) technique which uses the presence of a *Map* specific gene to confirm the positive result. To establish what role non-livestock species in Western Canada may serve in the spread and persistence of a contaminated environment, four rounds of samples were collected throughout the year from a variety of species including birds, rodents, etc. from each of the selected farms. During each round of sampling, a variety of species were trapped on the infected farms and up to 30 samples (fecal and/or tissue) were taken from them to determine if they are either infected or

spreading *Map*. Species of interest included wild rodents such as Richardson ground squirrels and mice as well as birds commonly found on cow-calf operations such as swallows and sparrows. Animals were trapped and euthanized in a humane manner and care was taken to avoid trapping endangered species.

Part Two: Environmental testing during calving season as an alternate herd test

Thirty herds (including the original 6 herds from Part One) were selected for this portion of the study. On these farms, herd prevalence was determined by collected fecal samples on approximately 100 cattle greater than 2 years of age and using pooled fecal culture (5 cattle per pool) using the same methods as above. Environmental sampling was conducted at the midpoint of the calving season for that herd. The samples were collected by the local veterinarian in the same manner as described in Part One.

Part Three: Environmental Persistence

One herd was selected for the persistence portion of this study due to having environmental areas known to be contaminated with detectable *Map*. The initial plan was to use sites identified as *Map* positive in the first round of sampling. This was not possible due to the very low level of environmental contamination found on the initial 6 selected herds in Part 1. There were very few positive environmental samples that allowed practical protection from recontamination. Three areas were identified as contaminated with *Map* on this farm during the calving season sampling and were re-cultured monthly once these areas were confirmed to be culture positive for *Map*. This was done to determine how long *Map* could survive in these relatively natural conditions.

Two samples per site were taken regularly until they were culture negative for two consecutive sampling periods.

Because of the small number of positive environmental samples and alternative experiment was devised to examine the persistence of Map survival in the environment. Four experimental field plots were established on a farm that was identified as Johnes positive. These plots were established in areas that cattle did not have access to for contamination. Two plots were set up at an exposed, unshaded site and two plots were set up within a mature forested pasture site with limited wind or direct sun exposure. Each site had one soil plot and one slurry plot. These plots were all contaminated with the feces collected from a mature cow with clinical Johne's disease. Samples from each plot were collected immediately after contamination and then at two weeks, one month and then monthly until three consecutive negative cultures were attained. Both sites were protected from recontamination from cattle.

RESULTS:

Part One: Environmental and Wildlife Seasonality

All of the herds selected (6/6) completed all 4 rounds of the required sampling and were included in the analysis. Of these 6 herds, 2 (33.3%; 95% Confidence Interval (CI) 0-74.7) were identified as actively Johne's positive by having at least 2 positive serological tests and 4 (66.7%; 95%CI 25.3-100) of 6 were identified as actively Johne's positive by having at least one positive pooled fecal culture. Individual herds had between 0.5% and 15.3% of the individual cattle test positive on the ELISA and between 0% and 52.0% of the pooled fecal cultures test positive. Comparisons of herd tests are summarized in Table 1.

Table 1. Comparison of the results of three alternative herd test methods

Herd #	Test Method		
	Pooled Fecal Culture*	Serum ELISA**	Environmental Testing***
1	+	-	-
2	-	-	-
3	-	+	-
4	+	-	-
5	+	+	+
6	+	-	+

* + herd = 1 positive pool (5 samples/pool)

** + herd = >1 positive serological test

*** + herd = 1 culture positive environmental sample

Only 1 out of 150 (0.7%; 95%CI 0-2.0) of the water samples was positive for *Map*. These samples consisted of: 48 samples from cattle waterers, 24 biofilm samples, 55 dugout samples, and 23 farm drainage samples. The positive culture was cultured from a dugout

sample collected in the summer round of sampling from herd #6. Only 1 of 268 (0.4%; 95%CI 0-1.1) of the environmental samples (non-water) were positive for *Map*. The single positive culture was from a calving pen sample collected in the fall round of sampling from herd #4. *Map* was not detected in the any of the samples from the remaining sites. In the four rounds of wildlife sampling 0 of 438 samples were positive for *Map* consisting of 202 bird samples and 236 rodent samples. This suggests that *birds* and rodents commonly found on cow-calf herds in Saskatchewan are not significantly impacted by *Map* at the level of contamination that is present on these farms. It was not possible to determine any seasonal effects on *Map* contamination of the environment or of wildlife due to the very low level of contamination that was found.

Table 2. Culture results of four rounds of environmental sampling on all farms

	Non Water Source Environmental Samples	Water Source Samples	All Environmental Samples
Fall	1/58	0/41	1/99
Winter	0/71	0/35	0/106
Spring	0/74	0/40	0/114
Summer	0/65	1/34	1/99
Total	1/268	1/150	2/418

Part Two: Environmental testing as an alternate herd test

Twenty-seven of the thirty herds selected (90%) completed all the required sampling and were included in the analysis. Of these 27 herds, 17 (63.0%; 95%CI 44.4-81.5) had at least one positive pooled fecal culture. Individual herds had between 0% and 60.0% of the pooled fecal cultures positive.

None of the 114 water samples (0%) were positive for *Map*. These included 54 samples from cattle waterers, 26 biofilm samples, 21 dugout samples, and 13 farm drainage samples

A very small percentage of the environmental samples (non-water) were positive for *Map*. Fifteen of the 243 samples collected (6.2%; 95% CI 3.1-9.2) were positively cultured for *Map*. Samples collected from within chutes were the most likely to be contaminated with *Map* with 4/26 (15.6%; 95%CI 1.2-29.5) positive. Other *Map* positive sites include: 3/21 (14.3%; 95%CI 0-29.6) of the samples from the ground outside of cow feeders, 2/21 (9.5%; 95%CI 0-22.4) of samples inside cow feeders, 2/13 (15.4%; 95%CI 0-35.8) of samples taken from mothering-up pens, 1/7 (14.3%; 95%CI 0-42.3) from bullpens, 1/10 (10.0%; 95%CI 0-29.6) from turnout pens, 1/18 (5.6%; 95%CI 0-16.4) from calf shelters, and 1/26 (3.8%; 95%CI 0-11.4) from calving pens. *Map* was not detected in the 101 samples from the remaining sites.

A total of 8 of the 27 infected herds (29.6%; 95% CI 12.1-47.2) had at least one environmental sample test positive for *Map*. Herds had between 0/15 (0%) and 3/9 (33.3%) of non-water environmental samples test positive for *Map*. Of the 17 herds identified as positive by pooled fecal culture, 6 (35.3%; 95%CI 11.9-58.7) were also

positive on environmental sampling. Of the 10 herds that were negative on fecal pools, 2 (20%; 95%CI 0-46.1) were positive on environmental sampling. The agreement between the two testing methods had a calculated kappa of 0.13 which suggests only slight agreement.

Part Three: Environmental Persistence

For the persistence study, 3 sites were identified as culture positive on a cow-calf farm in Saskatchewan not included in the rest of the study. These sites were all negative on culture when sampled again 5 and 6 months after the initial sampling. Because of the very small number of environmentally positive sites identified an alternative experiment was designed to provide further information on the persistence of *Map* in the environment. Four field plots have been experimentally contaminated with Map bacteria and will be cultured over time to evaluate persistence. These cultures are not yet completed and an addendum to this report will provide those results once they have been completed.

CONCLUSIONS AND RECOMMENDATIONS:

The level of environmental contamination was extremely low in Part One of the study with only 2/418 of all four rounds of sampling were positive for *Map*. The farms used in Part two of the study had slightly higher levels of contamination identified. However, none of the 114 water samples and only 15/243 (6.2%) of the non-water environmental samples collected during the calving season were positive for *Map*. This is likely due to a combination of multiple factors such as a relatively low prevalence of disease in the cow-calf industry and the extensive management practices of cow-calf farms including an relatively low cattle density. These results represent a much lower rate of environmental contamination than seen in dairy herds and it may be due to the fact that the animal populations are not as densely populated on cow-calf herds. Cow-calf herds also tend to have lower herd prevalences of Johnes disease infection and the herds identified for this study were no exception. It is not believed that seasonality played a significant role as no other season had a significantly higher rate of environmental contamination in Part One of the study. The higher *Map* positive rates around feeders and chute complexes suggest that these areas are not moved or cleaned frequently enough and may potentially be sites of increased risk of exposure to uninfected cattle and calves. The negative findings of hospital pens and manure storage samples were surprising. It may be that owners are recognizing cattle with clinical Johne's disease and not allowing them into the hospital pens or perhaps these pens are being adequately cleaned out between cattle and are therefore not as likely have positive samples collected. The manure storage areas may be negative due to the beneficial effect of composting as described by Grewal et al., 2006.

This low level of environmental contamination would suggest that cattle in cow-calf herds are relatively unlikely to become infected with *Map* by being exposed to an infected environment. Cow-calf producers who wish to reduce the level of Johnes disease in their herd should focus instead on identifying positive cows and removing them from the herd as soon as possible.

The low level of environmental contamination and lack of any positive wildlife samples on the cow-calf farms that were followed for a year made conclusions on significant seasonal effects impossible. The complete lack of positive samples from the wildlife samples suggests that at this time non-ruminant wildlife do not play a significant role in the epidemiology of Johne's disease on cow-calf farms.

The low level of contaminated samples and the low ability of the environmental sampling to identify herds as positive for *Map* suggest that environmental sampling as done in this project is not likely an adequate alternative herd test. Environmental sampling was only sensitive enough to identify 8/27 (29.6%) of the herds with a positive history of clinical Johne's disease and had only slight agreement (kappa = 0.13) with fecal pool sampling which may suggest that using individual animal tests on the entire herd may be the best option to determine the status of a replacement source herd. While reducing the cost of testing, using environmental samples as done in this study is not sensitive enough as a herd test to be used in place of traditional herd test methods. The infection levels on these farms were generally low and may be the reason that *Map* was not able to be detected more frequently in the environmental sampling. A few sites such as chutes, feeders, and mothering up pens were found positive on multiple farms

suggesting that if environmental testing was to be used there should be a focus on these sites in order to reduce cost.

The low level of contamination of environmental sites made the interpretation of the results of the persistence portion of this study difficult. This portion of the study did not find any *Map* surviving up to 5 months. This suggests that *Map* may not survive the natural conditions and climate present at these sites for durations up to a year in length as has been reported for other environments in previous papers (Whittington et al., 2004). This issue is presently being studied further through an experimental field study to improve confidence in the findings. This will enable samples to be collected regularly from the time of contamination until the sites are no longer culture positive. This will also allow for more specific results in the first 5 months of the study that were not possible in this pilot project.

The results of this study show that the level of environmental contamination of *Map* on cow-calf herds in western Canada is very low at this time. While continuing to maintain a hygenic environment is still strongly recommended, these results suggest that efforts to control this disease should be focussed on other management factors that can reduce the exposure of susceptible animals to *Map* such as not keeping infected animals on farm and reducing the movement of potentially infected animals onto the farm. The cow-calf industry has an opportunity to control this disease now before the prevalence of the disease increases in the population and before environmental contamination becomes a more significant challenge.

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Results from this study have been combined with results from concurrent research and presented at the Animal Determinants of Emerging Disease Rounds. These national rounds are hosted by the Centre for Coastal Health in British Columbia and involve veterinary and medical researchers from across Canada that are interested in emerging disease issues in animals and humans. The results of the study were submitted as an abstract to the American Association of Bovine Practitioners (AABP) and were accepted to be presented at the AABP Conference in September 2007 in Vancouver, British Columbia. This conference will play host to bovine veterinarians from across North America as well as veterinary researchers from around the world. The results of the study are currently being prepared for submission for publication in a peer reviewed journal as well as published as part of a PhD thesis for Dr. Dale Douma. The final expense statement is in the process of being generated by Mr. Calvin Mah of Financial Reporting at the University of Saskatchewan and will be submitted as soon as completed.